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DATA EVALUATION RECORD

STUDY TYPE: Developmental Neurotoxicity Study - Rat; OPPTS 870.6300 (§83-6); OECD 426 (draft)

PC CODE: 129140 **DP BARCODE:** D436931

TEST MATERIAL (PURITY): Transfluthrin Technical (99.1% a.i.)

SYNONYMS: NAK 4455; benfluthrin; (2,3,5,6-tetrafluorophenyl)methyl(1*R*,3*S*)-3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylate; Cyclopropanecarboxylic acid, 3-(2,2-dichloroethenyl)-2,2-dimethyl-, (2,3,5,6-tetrafluorophenyl)methyl ester,(1 R-trans)-

CITATION: Gilmore, R.G., Sheets, L.P., and Hoss, H.E. A Developmental Neurotoxicity Study with Technical Grade Transfluthrin in Wistar Rats. Bayer CropScience LP, Toxicology (Stilwell, Kansas). Laboratory Study Number 06-D72-DH. April 17, 2007. MRID 50130601. Unpublished.

SPONSOR: Bayer Environmental Science

EXECUTIVE SUMMARY:

In a developmental neurotoxicity study (MRID 50130601) transfluthrin (99.1% a.i., EATFTJ005) was administered to 30 female Wistar rats/dose in diet at dose levels of 0, 500, 2000, and 7000 ppm (equivalent to 0, 42.1, 161, or 534 mg/kg/day) from gestation day (GD) 6 through lactation day (LD) 21 with adjustments during lactation to maintain a more consistent dosage throughout exposure. Diet was provided for *ad libitum* consumption throughout the study, except during the neurobehavioral testing. Litters were culled to yield as closely as possible with four males and four females on postnatal day (PND) 4. Subsets of offspring were subjected to evaluation of detailed clinical observations and a functional observational battery, preputial separation or vaginal patency, body weight, automated measures of activity (figure-eight maze), auditory startle habituation, learning and memory (passive avoidance after weaning and a water maze task beginning on PND 60 ±2 days) and an ophthalmic examination. Neuronal tissues were collected from 10/sex/dietary level on PND 21 (brain only) and at study termination (PND 75±5 days) for microscopic examination and morphometry.

Maternal toxicity: There were no treatment related effects on survival or clinical signs for females in the P-generation. There was a 10% decrease in body weight gain at 534 mg/kg/day during GD 0-20 but this was attributed to decreased food palatability. However, there was no treatment-related effects on absolute body weight. There was no effect on reproductive performance or fertility index.

The maternal LOAEL for transfluthrin was not established. The maternal NOAEL is 7000 ppm (equivalent to 534 mg/kg/day).

Offspring toxicity: There was no treatment-related effect on litter parameters, pup viability, or clinical observations. Offspring body weights were statistically decreased in the high-dose females and males (>5%) on PND 11, 17, and 21. The ages for onset of balanopreputial separation, vaginal patency, sexual maturation, onset for surface righting and pupil constriction were not affected by treatment at any dietary level.

There were no compound-related effects on the FOB or measures of motor or locomotor activity for both sexes at any dietary level. Startle amplitude, latency, and habituation were not affected by treatment on any test occasion.

Absolute brain weight for non-perfused terminal males was not affected at any dietary level. There were no compound-related necropsy findings in animals that were either found dead or sacrificed on PND 21 or at study termination. For the micropathology brain measurements, the hippocampus thickness for the high-dose females (1.4 mm) was statistically decreased compared to the controls (8%) at PND 75 and was smaller than both the concurrent (1.56 mm) and historical (1.48-1.76 mm) controls; however, this decrease was not toxicologically adverse due to no effects on functional activity (learning and memory). There were no other effects of treatment on brain measurements.

The offspring LOAEL for transfluthrin is 7000 ppm (equivalent to 534 mg/kg/day) based on decreased pup body weight in males and females (>5%). The offspring NOAEL is 2000 ppm (equivalent to 161 mg/kg/day).

This study is classified acceptable and satisfies the guideline requirement for a developmental neurotoxicity study in rats (OPPTS 870.6300, §83-6); OECD 426 (draft). The study was conducted with a few deviations to the guidelines outlined in the 1999 Data Call-In (DCI) Notice issued for several organophosphorus insecticides. The modifications include: (1) extend exposure to lactation / postnatal day 21 (rather than day 11) and (2) evaluate brains from 21-day-old (rather than 11-day-old) animals for morphometry and micropathology using (3) a sample size of 10 (rather than six) per sex per dietary level. These design elements are consistent with the draft OECD guideline for a Developmental Neurotoxicity Study (TG 426).

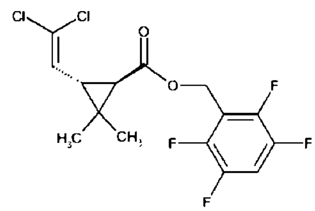
COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Transfluthrin Technical

Description: Colorless liquid
Lot/batch #: EATFTJ005
Purity: 99.1% a.i. (May 2004); Expiration: May 2006
Stable at room temperature (25 ± 5°C)
Compound stability: Concentration of a.i. in the test substance was measured before initiation of dietary exposure in order to verify stability at the room temperature storage conditions used in this study.
CAS # of TGAI: 118712-89-3
Structure:



2. Vehicle and/or positive control: Diet

3. Test animals (P)

Species: Rat
Strain: Wistar Crl:WI(Han)
Age at study initiation: At least 15 (males) or 12 (females) weeks of age at co-housing
Wt. at study initiation: Females: 198.6-249.1 g; Males have no specified weight requirements.
Source: Charles River Laboratories, Inc., Raleigh, NC
Housing: Suspended stainless steel cages; Individually, except with one male each during cohabitation with deotized (sanitized) cage board in the bedding tray. Individually in plastic cages with corn cob bedding during gestation and lactation. Each cage contained a feeder and a source of water (pressure-activated water nipples).
Diet: Purina Mills Certified Rodent Diet 5002 in meal form provided for *ad libitum* consumption during the acclimation period and throughout the study except during neurobehavioral testing.
Water: Tap water (Kansas City Missouri Municipal Water) was provided *ad libitum* except during neurobehavioral testing.
Environmental conditions:

Temperature:	18-26°C[1]
Humidity:	30-70% (relative)
Air changes:	Minimum daily average 10.33 per hour.
Photoperiod:	12 hrs light/ 12 hrs dark; lights toggled off during ophthalmic examinations.

Acclimation period: 6 days

B. PROCEDURES AND STUDY DESIGN

1. In life dates:

The First Study Specific Data Were Collected:	January 9, 2006
The Test Substance was First Applied to Test System:	January 22, 2006
Experimental Completion Date:	April 21, 2006

[1] *On 4/7/06, the high temperature and high relative humidity in animal room 301 were recorded as 99 °F and 99%, respectively. It is highly unlikely that these readings represent actual temperature and humidity levels, but were caused by an error in data collection or transmission. This deviation did not adversely impact the outcome of the study.*

2. **Study schedule:** The maternal animals (P-generation) were mated and assigned to study when they were determined to be sperm positive. The test substance was administered via the feed from gestation day 6 through day 21 of lactation / postnatal development. On postnatal day (PND) 21, pups were weaned and maternal animals were sacrificed. The offspring (Fl-generation) that remained on study after weaning were sacrificed at study termination on PND 75 (±5 days).

3. **Mating procedure:** Mating was managed by co-housing one female with one male in suspended stainless-steel gang cages for a maximum of five consecutive days. The dams and cages were examined each morning during the co-habitation phase for a vaginal plug. Vaginal smears were taken and examined for the presence of sperm. The day on which insemination was observed in the vaginal smear was designated day 0 of gestation (GD 0) for that female, and the female was removed and housed individually in a plastic nesting cage. Typically, females that were not sperm positive were sacrificed without a necropsy examination.

4. **Animal assignment**: Upon receipt, P-generation females were examined acclimated to ambient laboratory conditions for at least six days prior to the study. For the holding period, the animals were observed at least once daily for moribundity and mortality. Moribund animals were sacrificed. Several males and females were subjected to a gross necropsy and serological examination for sentinel animal/vendor surveillance evaluation. Dams were assigned to functional observational testing as shown (Table 1). Male animals were randomly assigned an identification number upon arrival when they were arbitrarily selected, removed from the shipping crates, and placed into individual cages. Following at least six days of acclimation, P-generation females were weighed, and the females with a body weight more than ±20% of the mean weight were rejected. The remaining females were assigned to the control or an exposure group in sequence, as they were determined to be inseminated to ensure unbiased assignment of the animals to dose groups and that there would be approximately an equal number of litters from each dose group available for testing on a given day. Females that were not placed on study were sacrificed without necropsy. Animals were assigned an identification number that specified the sex, dietary level, cage number and identified it with the study. P-generation males served only as breeders, thus they had no specific weight requirements and were arbitrarily selected for co-housing with females. Parental (P)-generation males and females were identified by cage card and tail mark (males) or tail tattoo (females). Fl-generation animals that were born alive were identified by tattoo; pups that were found dead were identified with a marking pen.

   Offspring were assigned to testing subgroups at the time of litter standardization on postnatal day 4 (Table 1). An animal allocation program was used to assign offspring to four sets (designated A-D) for assessment at each age. One male and/or female per litter (approximately 16 (minimum 10)/sex/dietary level, representing at least 20 litters per level): Motor activity (Set A), Auditory Startle (Set B), Passive Avoidance, Water Maze and Functional Observational Battery (Set C). On PND21, the whole brain was collected from a separate group of randomly selected offspring (Set D; 10/sex/dietary level; representing 20 litters per level) for micropathologic examination and morphometric analysis. The remaining pups assigned to Set D (~ 10/sex/dietary level) were reserved for possible use as replacement animals and sacrificed on PND21 without necropsy examination. At approximately 50-60 days of age, randomly selected animals (a minimum of 10/sex/dietary level, representing at least 20 litters per level) from Sets A, B and C were subjected to an ophthalmologic examination. At termination (PND75 [± 5 days]), these animals were anesthetized and sacrificed by perfusion, with neural and muscle tissues collected for microscopic examination. At termination (PND75 [±5 days]), brains were collected from additional randomly selected animals (10/sex/dietary group; representing 20 litters per level). These brains were weighed (fresh tissue weight) and then discarded. The remaining animals assigned to sets A-C were sacrificed without routine gross necropsy examination or collection of tissues.

TABLE 1. Study Design

Experimental parameter	Dose (mg/kg/day)			
	0	**42.1**	**161**	**534**
Maternal animals				
No. of maternal animals assigned	30	30	30	30
FOB (GD 13, 20/LD 11, 21)	30/9	30/10	30/10	30/10
Offspring				
Detailed clinical/FOB (PND 4, 21, 35(±1), 45(±1), 60(±2))	19-20 (min 10)/sex	20 (min 10)/sex	20 (min 10)/sex	20 (min 10)/sex
Motor activity (PND 13, 17, 21, 60(±2))	19-20 (min 10)/sex	20 (min 10)/sex	19-20 (min 10)/sex	19-20 (min 10)/sex
Auditory startle habituation (PND 23, 60(±2))	19-20 (min 10)/sex	19-20 (min 10)/sex	20 (min 10)/sex	20 (min 10)/sex
Learning and memory (PND 23/30, 60(±2)/67(±2))	16 (min 10)/sex	16 (min 10)/sex	16 (min 10)/sex	16 (min 10)/sex
Brain weight				
PND 21	10/sex	10/sex	10/sex	10/sex
PND 75(±5)	10/sex	10/sex	10/sex	10/sex
Neuropathology				
PND 21	10/sex	10/sex	10/sex	10/sex
PND 75(±5)	10/sex	10/sex	10/sex	10/sex

Note: The method of animal assignment minimized potential problems related to litter effects, by using at least one pup/litter. For FOB and motor activity testing, the same individual animals were evaluated at all scheduled time points. For the selection of animals and testing paradigms for cognitive (learning and memory) assessment, the same animals were used for assessments at the weanling and adult ages, but different tests were used at the two ages.

5. **Dose selection rationale:** Dose levels were selected based on the results of a two-generation reproduction toxicity study and an oral subchronic toxicity study, which both used Wistar rats. In the two-generation reproduction study, rats were exposed to technical-grade transfluthrin at dietary levels of 0, 20, 200, or 1000 ppm. At 1000 ppm, there was slight evidence of toxicity, including effects on the liver and kidney in P-generation females, but there was no overt evidence of toxicity. In the subchronic oral toxicity study, animals received transfluthrin in the diet at 0, 10, 50, 500, or 5000 ppm for 13 weeks. Hepatocellular degeneration and degeneration of the proximal tubules was observed at 5000 ppm; however, there was no effect on body weight, feed consumption, or compound-related clinical signs at any dietary level.

   Based on these studies, a pilot study was conducted to determine whether 5000 ppm would be a suitable dose for a DNT study and whether residues of the test substance could be detected in tissues of the offspring at sufficient concentrations to verify exposure during lactation. In this study, eight pregnant Wistar rats were treated via the diet at 5000 ppm transfluthrin from GD 6 through LD 21, with adjustments in dietary level during lactation to maintain a more constant dosage (mg/kg/day) throughout exposure. Offspring from each litter were sacrificed on lactation days 10, 14, and 16 to measure the concentration of transfluthrin in the brain. There were no compound-related effects apparent in the dams or offspring. There were no detectible residues in the brain on PND 10 and very low residues on PND 16.

A second pilot study was conducted to test whether a higher dietary level of 7000 ppm transfluthrin could be detected in liver and kidney tissues to provide evidence of pup exposure to transfluthrin during lactation. Six pregnant Wistar rats were exposed to a nominal concentration of 7000 ppm transfluthrin in the diet from gestation day 7 through lactation day 21, with adjustments in dietary levels during lactation to maintain a more constant dosage (mg/kg/day) throughout exposure. Offspring from each litter were sacrificed on lactation days 10, 14, 18, and 21 (one/sex/litter at each age) to measure the concentration of transfluthrin in the liver and kidney. Tremors were observed in three dams – beginning on lactation day 3 in two animals and lasting up to five days. For all ages, transfluthrin was detected in the liver and kidney (Table 2), with higher concentrations in the kidney (2.5-9.4-fold higher). These data demonstrate that offspring were exposed to transfluthrin via the milk and to higher levels at the end of lactation when they were exposed through treated diet and milk.

Table 2. Average concentration (ppm) of transfluthrin [a]

Tissues	PND 10	PND 14	PND 18	PND 21
Liver	0.0164	0.0071	0.0065	0.0142
Kidney	0.0417	0.038	0.0271	0.1341

[a] Data obtained from page 21 from MRID 50130601.

Based on the results of the two pilot studies, the dietary levels for the developmental neurotoxicity study were 0, 500, 2000, and 7000 ppm, with adjustments during lactation to maintain a more constant dosage throughout exposure. The 7000 ppm dietary level was selected as a maximum dose the animals will tolerate without excessive toxicity. The 2000 ppm dietary level was selected as an intermediate dose that may produce effects that can be compared to the reproduction study and to assist in establishing compound-related effects. Finally, the 500 ppm dietary level was selected since it was expected to establish a NOAEL.

6. **Dosage administration:** All doses were administered once daily to maternal animals orally by treated feed, from GD 6 through LD 21.

7. **Dosage preparation and analysis:** Formulations were prepared weekly by mixing appropriate amounts of test substance with Purina Mills Certified Rodent Diet 5002 in meal form and were stored in the freezer at -23.0 to -24.0 ˚C. Dietary concentrations were not adjusted to correct for purity (percent active ingredient) in the test substance but were adjusted (reduced) during lactation, relative to gestation, to maintain a more constant level of exposure (mg/kg/day).

   Prior to the start of the study, stability of the test substance in feed was evaluated for a period of 7 days at room temperature and 49 days at freezing conditions. Homogeneity (top, middle, and bottom) was evaluated once prior to the initiation of this study. During the study, samples of each batch feed were analyzed to establish dietary concentrations.

   Results

Homogeneity analysis: The concentrations of 10, 5000, and 10000 ppm had percent relative standard deviations (%RSD) of 0.72%, 1.6%, and 1.1%, respectively.

Stability analysis*:* There was no appreciable decrease in concentration with 7 days of storage at room temperature or 49 days of storage at freezing conditions.

Concentration analysis*:* During week 2, the control diet was found to be contaminated with the test article (0.0649 ppm), which was likely from the equipment used during mixing. However, the contamination was an insignificant amount (0.01% of the low feed level) and only happened during one week of the study. Therefore, this contamination did not impact the reliability of the study.

During gestation, the mean concentration in the 500, 2000, and 7000 ppm test diets was 496, 2001, and 6972 ppm, respectively (99-100% of the nominal concentration). For lactation, dietary levels were adjusted to achieve a more consistent dosage (mg/kg/day) throughout the period of exposure, since food consumption increases during this time period. During lactation, the mean concentrations were 96-99% of the nominal concentrations.

The analytical data indicated that the mixing procedure was adequate and that the difference between nominal and actual dosage to the study animals was acceptable.

C. OBSERVATIONS

1. In-life Observations

a. Maternal Animals: Once daily checks for mortality or moribundity and daily cage-side observations were conducted for maternal animals. Gross observations of the dams were conducted daily, prior to treatment. Signs of toxicity were recorded as they were observed, including the time of onset, degree, and duration. Detailed clinical evaluations were conducted once daily during exposure (GD 6 through LD 21).

A functional observation battery was conducted on animals that were presumed to be pregnant (approximately 30/dietary level) on GD 13 and GD 20 and a minimum 10 dams/dietary level on LD 11 and LD 21 for dams that were maintained on study with suitable litters. All observations were performed by an individual who was unaware of each animal's dose group assignment. It was not feasible for one person to evaluate all animals on all test occasions. The laboratory maintains evidence of inter-observer reliability (agreement) for individuals who were involved with performing these observations. This evaluation was performed under standard animal room conditions (temperature, relative humidity, etc.) and included the following observations:

FUNCTIONAL OBSERVATIONS	
X	Signs of autonomic function, including: 1) Ranking of degree of lacrimation and salivation, with range of severity scores from none to severe 2) Presence of absence of piloerection and exophthalamus, 3) Ranking or count of urination and defecation, including polyuria and diarrhea 4) Pupillary function such as constriction of the pupil in response to light, or a measure of pupil size 5) Degree of palpebral closure, e.g., ptosis.
X	Description, incidence, and severity of any convulsions, tremors, or abnormal movements.
X	Description and incidence of posture and gait abnormalities.
X	Description and incidence of any unusual or abnormal behaviors, excessive or repetitive actions (stereotypies), emaciation, dehydration, hypotonia or hypertonia, altered fur appearance, red or crusty deposits around the eyes, nose, or mouth, and any other observations that may facilitate interpretation of the data.

Individual maternal body weight data were recorded once weekly throughout gestation and lactation, as follows: GD 6-13, GD 13-20 and LD 0-7, 7-14, and 14-21. Dams were also weighed on LD 4. Food consumption measurements may have included consumption by the pups. Fresh feed and clean feeders were provided weekly.

b. **Offspring**

1. **Litter observations:** The day of completion of parturition was designated as LD (PND) 0. Live pups were counted, sexed and weighed individually for each litter on PND 0, 4, 11, 17, and 21. Daily throughout lactation, offspring were examined cage-side for gross signs of mortality or morbidity. Any gross signs of toxicity in the offspring were recorded as they were observed, including the time of onset, degree, and duration. More detailed observations for clinical signs were made once daily (a.m.) before weaning and once weekly thereafter. These observations were performed by an individual who was aware of assignments to dose level.

2. **Developmental landmarks:** Beginning on PND 38, male offspring were examined daily for balanopreputial separation. Beginning on PND 29, female offspring were examined daily for vaginal patency. The age of onset was recorded. Beginning on PND 4, selected males and females were examined daily for surface righting. The age of onset was recorded. On PND 21, all pups were also tested for the presence of pupil constriction.

3. **Post-weaning observations:** After weaning on PND 21, offspring were examined twice daily for mortality, and cage-side observations were conducted once daily. Individual offspring body weight data were recorded weekly as well as on the day that vaginal patency or balanopreputial separation was achieved.

4. **Neurobehavioral evaluations:** The test room used for motor activity, auditory startle habituation and passive avoidance conditioning was a standard animal room that was maintained on the same light: dark cycle as the room in which animals were housed, with tests conducted during the light phase. The water maze testing was performed in the room where animals were housed. The order of testing and assignment of animals to specific test devices was semi-random, such that groups were balanced across test times and

devices, and no animal was tested more than once in the same device. One planned exception was that animals were purposely tested in the same water maze on both occasions, as per standard procedure. Males and females were generally tested on the same days at the appropriate days of age. After sexual maturation, test devices were cleaned during the ensuing interval to reduce the residual scent from the other gender

i. **Functional observational battery (FOB):** On PND 4, 11, 21, 35, 45, and 60, a total of 10 offspring/sex/group (one male or one female from each litter) was examined outside the home cage in an FOB assessment, as appropriate for the developmental stage being observed. The same parameters assessed in the maternal FOB were examined for offspring except for neonates (PND 4 and 11) were not evaluated in the open field, as the observer did not consider it necessary.

ii. **Motor activity testing:** Motor activity was evaluated in approximately 20 rats/sex/dose on days 13, 17, 21, and 60 (±2 days). The same offspring were evaluated in the figure-eight maze for 60 minutes at each time point, using a computer-automated system (Columbus Instruments, Columbus, OH) and personal computer for automated data collection. Motor and locomotor activities were examined as total activity counts (beam interruptions) for the 60-minute session and as activity during each ten-minute interval. Motor activity was measured as the number of beam interruptions that occurred during the test session. Locomotor activity was measured by eliminating consecutive counts for a given beam, so only one interruption of a given beam was counted until the rat relocated in the maze and interrupted a different beam. Habituation was evaluated as a decrement in activity over consecutive intervals of the test session.

iii. **Auditory startle reflex habituation:** Auditory startle reflex habituation testing was performed on 10 offspring/sex/dose on PND 22 and 60 (±2 days), using an automated system. A personal computer was used to control the operation of an integrated startle response test system (Coulbourn Instruments, Allentown, PA) and for automated data collection. Groups of four animals were tested simultaneously within each of the two startle system enclosures. Each enclosure was ventilated, lined with sound-attenuating and vibration-absorbing material, and houses a speaker mounted in a central position within the ceiling of the enclosure to provide the eliciting stimulus (S2) - a 50-msec burst (0 msec rise/fall) of broad-spectrum "white" noise [approximately 118 dB(lin)]. Four load cell/force transducer assemblies designed to measure the startle response were in each enclosure. The test session consisted of 50 trials that began following an approximately 5-minute adaptation period at ambient noise levels. The rats were then presented with the startle-eliciting stimulus at 10-sec intervals. The average response amplitude and the magnitude of decrease (habituation) over blocks often trials were compared among the dosage groups. Data collection began with the presentation of S2 and continued thereafter for 200 msec. The analog signal for each response output (measured in mV) was digitized at one kHz (i.e., one sample/msec for 200 msec) and converted to grams using a previously determined calibration curve for each load cell. Peak response amplitude (g) and latency (msec) measurements were taken from each animal's individual response curve. Baseline was defined as the average force (g) exerted on the platform during the first 8 msec following the onset of S2, a time period that precedes response onset and represented an approximate body weight measurement that was used to verify that the equipment used to measure the response amplitude was functioning properly. Response amplitude is defined as the maximum value of the average curve, minus the baseline (i.e., removing the animal's body weight from the measurement). Latency to peak is the time (msec) following the onset of S2 when the peak response amplitude occurs.

iv. **Learning and memory testing:** Learning and memory testing was performed in 16 offspring/sex/dose (minimum of 10 offspring/sex/dose). The same set of animals were used for testing passive avoidance (on PND 23 and 30) and water maze (PND 60±2 days and again 7 days later).

Post-weaning – Passive avoidance: Animals were tested for acquisition on PND 23 and for retention on PND 30. Testing was conducted using equipment and computer programs from Coulbourn Instruments (Allentown, PA). A personal computer was used to control the operation of the equipment and for automated data collection. Testing took place in individual isolation cubicles, each housing a single shuttle cage. Each isolation cubicle was lined with foam insulation to attenuate sound in the chamber and had a fan with a baffled air intake and exhaust system for ventilation. The shuttle cage consisted of a Plexiglas and stainless-steel rectangular chamber fitted with front-loading access. Each shuttle cage (15 inches wide x 7.25 inches deep) was separated into two compartments of equal size (approximately 7 x 7 inches) by a wall that supported a centrally-located sliding (guillotine-type) door. One compartment was lined with black film (dark-side), while the other side was illuminated during the test with a high intensity lamp. The lamp was turned on at the start of each trial and remained on until either the animal crossed to the dark compartment or the trial ended. The floor of the cage consisted of a grid of stainless-steel bars. The movement of the animal from the starting (light) side to the dark compartment was detected by a photocell system. A Coulbourn solid-state scanning shock generator was used to deliver a brief (0.5 sec) pulse of mild (0.5 mA) distributed shock to the grid floor when the animal crossed to the dark compartment. After adaptation, individual animals were place individually into the "lighted" compartment of a conditioning apparatus (the shuttle cage), facing toward the light. After approximately 60 seconds, the trial began with the light being illuminated to signal the beginning of the trial and the door separating the two compartments opening, so that each rat was provided access to the non-illuminated side of the cage. When the rat crossed into the dark compartment, the door automatically closed, the shock was delivered, and the light switched off - signaling the end of that trial. At that time, the animal was returned promptly to the holding cage to wait for the next trial. If the rat failed to cross within 180 sec, it was returned to the holding cage and the latency assigned an arbitrary score of 180. The procedure was repeated until either the rat remained in the lighted compartment for 180 sec on two consecutive trials or until 15 trials had elapsed, whichever occurred first, and these animals were excluded in the retention phase. Rats that failed to meet the criterion during the learning phase were assigned a value of 15 for the trials-to-criterion variable. The test was repeated one week later. For this second trial, rats were placed in the illuminated side of the apparatus, given a 20-sec acclimation period, and the latency to enter the dark side recorded.

Adult (PND 60) Offspring - Water maze: Animals were tested on PND 60 (+2 days), and again seven days later. Only animals that demonstrated acquisition were tested for retention. The water in the M-maze was maintained at 22 +1 °C. The mazes were constructed of opaque Plexiglas, with corridors approximately five inches wide and walls approximately 16 inches high with approximately 7.5 inches of water. On each test trial, the rat was placed into the starting position at the base of the M-maze stem, located between the two lateral arms. On the first (learning) trial, the rat was required to enter both arms of the maze before being provided access to the exit ramp to escape the water and then removed from the maze. The initial arm chosen on this learning trial was designated the incorrect goal during the subsequent 15 trials (maximum). Rats that failed to make correct goal choice within 60 seconds in any given trial were guided to the correct goal with the exit ramp and then removed from the water. Between trials, the animal was returned to a transport cage to wait for the next trial. The inter-trial interval was approximately 15 (+5) seconds. Each rat was required to reach a criterion of five consecutive error-less trials to terminate the test session. Latency (in seconds) to choose the correct goal or the maximum 60-second interval and the number of incorrect turns in the maze were recorded for each trial. Animals that satisfied the above criteria within the 15-trial limit were tested for retention seven days following acquisition. The correct goal and the criterion were the same for both sessions.

5. **Ophthalmology:** At approximately 50-60 days of age, ophthalmic exams were conducted using the males and females (a minimum of 10/sex/dietary level; representing at least 20 litters per level) that were selected for perfusion at study termination. The animals reserved for adult brain weight measurements were also subjected to ophthalmologic examination. The exam took place in a semi-darkened room. The pupillary reflex was tested using a penlight or transilluminator, with a mydriatic agent applied to each eye to dilate the pupil. The conjunctiva, cornea and lens were examined with a slit lamp microscope either before or after pupillary dilatation. After mydriasis, the vitreous humor, retina, choroid, and optic disc were examined using an indirect ophthalmoscope equipped with a condensing lens.

2. Postmortem observations

a. **Maternal animals:** Maternal animals were sacrificed by CO_2 asphyxiation on LD 21 following the weaning of their respective litters and discarded without postmortem examination. Females that were sperm positive and/or had an internal vaginal plug but did not deliver were sacrificed on GD 24 without necropsy examination.

b. **Offspring:** The offspring selected for brain weight or neuropathological evaluation were sacrificed on PND 21 or 75 (±5 days). These animals were subjected to postmortem examinations as described below.

At PND 21 (from Set D) or PND 75±5 (from Sets A-C), ten pups/sex/group were selected for brain weight measurements. The brain was weighed upon removal from the skull, prior to placement into formalin, and the brain:body weight ratio calculated. The brains from 10 of these pups/sex/group were post-fixed in 10% buffered formalin and embedded in paraffin, blocked, sectioned, and stained with hematoxylin and eosin. The brain was then divided into eight coronal sections for microscopic examination and processed according to standard procedures for paraffin embedding, sectioned at approximately 5 µm, and examined after staining with hematoxylin and eosin (H&E). In addition, the brain sections reserved for morphometric measurements (levels 3-5 and 7) were stained using luxol fast blue/cresyl violet. Histopathological examination was performed on tissues from control and high-dose pups. Detailed morphometric evaluation of the neocortex, hippocampus, cerebellum, three levels of the spinal cord (cervical, thoracic and lumbar), the cauda equina, eyes, optic nerves and gastrocnemius muscle was conducted. Dorsal root ganglia (including dorsal and ventral root fibers) from the cervical and lumbar swellings and gasserian ganglia were embedded in glycol methacrylate (GMA). GMA-embedded tissues were sectioned at 2 µm – 3 µm and stained using a modified Lee's stain. Peripheral nerve tissues (sciatic, tibial and sural nerves) were embedded in GMA resin and sectioned longitudinally. The sciatic nerve was also cut in cross section.

On PND 21 (from Set D) or PND 75±5 (from Sets A-C) 10 animals/sex/group were euthanized and perfused via the left ventricle with a sodium nitrite (in phosphate buffer) flush followed by in situ fixation using universal fixative (1.0% (w/v) glutaraldehyde and 4% (w/v) EM-grade formaldehyde) in phosphate buffer for brain weight measurements and/or neuropathology. On PND 21, only the brain (with olfactory bulbs) was collected. At PND 75±5, the brain and spinal cord, both eyes (with optic nerves) and selected (bilateral) peripheral nerves (sciatic, tibial and sural), the gasserian ganglion, gastrocnemius muscle, both forelimbs and physical identifier were collected. The brain tissue from perfused animals, and any gross lesions collected at necropsy, were further processed for microscopic examination.

The CHECKED (X) tissues were evaluated for adult offspring.

	CENTRAL NERVOUS SYSTEM		PERIPHERAL NERVOUS SYSTEM
	BRAIN		**SCIATIC NERVE**
X	Forebrain		Mid-thigh
X	Center of cerebrum		Sciatic notch
X	Midbrain		
X	Cerebellum		**OTHER**
X	Pons	X	Sural nerve
X	Medulla oblongata	X	Tibial nerve
	SPINAL CORD		Peroneal nerve
X	Cervical swelling	X	Lumbar dorsal root fibers
X	Lumbar swelling	X	Lumbar dorsal root ganglion
X	Thoracic	X	Lumbar ventral root fibers
	OTHER	X	Cervical dorsal root ganglion
X	Gasserian ganglion	X	Cervical dorsal root fibers
	Trigeminal nerves		Cervical ventral root fibers
X	Optic nerve	X	Gastrocnemius muscle
X	Eyes		
X	Cauda equina		

D. <u>DATA ANALYSIS</u>

1. **Statistical analyses:** Statistical evaluations were generally performed using software from INSTEM Computer Systems, SAS or TASC. The level of significance was set at $p \leq 0.05$, with the exception of Harriett's test, which was tested at $p<0.01$. In general, continuous data were initially assessed for equality of variance using Bartlett's test. Group means with equal variances were analyzed further using an Analysis of Variance (ANOVA), followed by a Dunnett's test if a significant F-value was determined in the ANOVA. In the event of unequal variances, these data were analyzed using nonparametric statistical procedures (Kruskal-Wallis ANOVA followed by the Mann-Whitney U test for between-group comparisons). **Functional Observational Battery**. Continuous data were analyzed using an ANOVA, *with post-hoc* comparisons using Dunnett's test. Categorical data were analyzed using General Linear Modeling and Categorical Modeling (CATMOD) Procedures, *with post-hoc* comparisons using Dunnett's test and an Analysis of Contrasts, respectively. **Motor and locomotor activity** (total session activity and activity for each 10-minute interval) were analyzed using ANOVA procedures. Session activity data for the four test occasions were first analyzed using an ANOVA to determine whether there was a significant day by treatment interaction. For days on which there was a significant treatment effect, Dunnett's test was used to determine whether the treated group was significantly different from the control. Interval data were subjected to a Repeated-Measures ANOVA, using both test interval and test occasion as repeated measures, followed by an ANOVA to determine whether there was a significant treatment by interval interaction on each test occasion. For those test days, the data for each interval was subjected to analysis using Dunnett's test to determine whether the treated group was significantly different from the control. **Auditory startle** response amplitude data (peak amplitude) for the two test occasions were first analyzed using an ANOVA procedure. If there was a significant group effect, Dunnett's test was used to determine whether the treated group was significantly different from control. The response amplitude data for each block often trials (five blocks/test session) were subjected to a Repeated-Measures ANOVA, using test block as the repeated measure. If there was a significant group by block interaction, the values for each block were subjected to analysis using Dunnett's test to determine if the results for treated animals were significantly different from control. **Passive avoidance** data were analyzed as follows. Latency data were analyzed using a Wilcoxon Test for time to failure (i.e., time to cross). The number of trials-to-criterion was analyzed using Kruskal-Wallis and Wilcoxon tests for the acquisition phase and Fisher's Exact Test for retention. The number of rats failing to meet the criterion level of performance in the learning (acquisition) phase was analyzed as incidence data. **Water maze** results were analyzed using parametric and non-parametric tests. Latency data were analyzed by a univariate ANOVA, with post-hoc analysis using Dunnett's test. The number of trials-to-criterion and the number of errors were analyzed using Kruskal-Wallis and Wilcoxon tests for the acquisition phase and Fisher's Exact Test for retention. The number of rats failing to meet the criterion level of performance in the learning phase was analyzed as incidence data. **Pathology** data were screened for potential effects and then evaluated using the following approach. Additional statistical tests to assess continuous and frequency data may have been used when deemed appropriate.

2. **Indices**

   a. **Reproductive indices:** The following reproductive indices were calculated from breeding and parturition records of animals in the study:

$$\text{Mating Index} = \frac{\text{No. of inseminated females}}{\text{No. of females co} - \text{housed with males}} \text{ x } 100$$

$$\text{Fertility Index} = \frac{\text{No. of pregnant females}}{\text{No. of inseminated females}} \text{ x } 100$$

b. **Offspring viability indices:** The following viability (survival) indices were calculated from lactation records of litters in the study*:*

$$\text{Live Birth Index} = \frac{\text{No. of live pups born per litter}}{\text{Total no. of pups per litter}} \text{ x } 100$$

$$\text{Viability Index} = \frac{\text{no. of live pups on day 4 pre} - \text{culling per litter}}{\text{No. of live pups born per litter}} \text{ x } 100$$

$$\text{Lactation Index} = \frac{\text{No. of live pups on Day 21 per litter}}{\text{No. of live pups on day 4 post} - \text{culling per litter}} \text{ x } 100$$

3. **Positive and historical control data:** Concurrent positive controls were not included in the study, but references were made to previous studies as controls. Positive control data demonstrated sensitivity to the test method to detect changes in the measured parameters. These data were generated from both prenatal exposure and young-adult rats that collectively verify the laboratory competence in evaluation of effects in neonatal animals perinatally exposed to chemicals and establish test norms for the appropriate age group. For observational measures, the data demonstrate the ability to detect major neurotoxic endpoints, including limb weakness, tremor, and autonomic signs; motor activity positive control data demonstrate the ability to detect both increases and decreases in motor activity. Pathology positive control data demonstrate the ability to detect central and peripheral nervous system pathology (separate groups were used to demonstrate each type of pathology, using acrylamide for peripheral nervous system pathology and trimethyl tin for central nervous system pathology). The methods were the same as those used in the study. Statistical evaluations used were the same as those used in the present study, and the number of animals per test group was not greater than that used in the study being evaluated. Positive control data demonstrate inter-observer reliability for the FOB. The positive control were collected within a reasonable time frame (e.g., the last few years). New data are collected when observational personnel or other critical laboratory elements change.

   It was not feasible for one person to evaluate all animals on all test occasions. The laboratory maintains evidence of inter-observer reliability for individuals that were involved in observations. For measures of activity in the figure-eight maze, studies with untreated rats and rats treated reference chemicals that increase (triadimefon) and decrease (chlorpromazine) activity were conducted to verify the sensitivity, reliability, and validity of the test procedures. The adequacy of the auditory startle test procedures has been established by performing studies with untreated animals and with rats treated with reference substances (8-OH-DPAT and mCPP) that alter startle response amplitude. The adequacy of the passive avoidance test procedures has been established by performing studies with untreated animals and with rats treated with a reference substance (scopolamine) that interferes with acquisition and/or retention. The adequacy of the water maze test procedures was also established by performing studies with untreated animals and rats treated with a test substance (scopolamine) that interferes with acquisition and/or retention.

II. RESULTS

A. PARENTAL ANIMALS

1. **Mortality and clinical and functional observations:** No P-generation females or males were found dead after the initiation of the study. All findings were considered incidental and unrelated to treatment.

2. **Body weight and food consumption:** Selected group mean body weights and food consumption values for pregnant or nursing dams are summarized in Table 2.

   There were no treatment-related adverse effects on absolute body weight for any dietary level during gestation or lactation. During gestation (GD 0-20), body weight gain was reduced 10% for the high-dose dams compared to the controls; however, this was thought to related to excessive feed spillage, which could indicate reduced feed palatability.

TABLE 2. Mean (±SE) maternal body weight and food consumption [a]

Observations/study week		Dose (mg/kg/day)			
		Control	42.1	161	534
Gestation					
Mean body weight (g)	GD 0	231.9±2.34	226.2±1.75	230.3±2.33	228.4±2.63
	GD 6	251.5±3.06	244.5±1.51	246.6±3.97	248.4±2.85
	GD 13	276.4±3.72	269.0±2.10	273.8±3.28	267.7±3.09
	GD 20	337.1±5.28	328.3±2.95	330.1±4.39	323.2±3.45
Body weight gain (g)	GD 0-20	105.2±3.94	102.1±2.53	99.8±2.96	94.8±2.22 (↓10%)
Mean food consumption (g/animal/day)	GD 6-13	22.7±0.78	22.0±0.77	20.9±0.59	42.3±4.67**[b] (↑86.3%)
	GD 13-20	23.5±0.71	23.9±0.80	22.5±0.66	22.3±0.81
Lactation					
Mean body weight (g)	LD 0	267.2±3.98	260.5±3.03	265.3±3.50	253.5±3.14* (↓5.1%)
	LD 4	284.0±4.31	272.5±3.27	277.6±4.39	267.3±3.91* (↓5.9%)
	LD 7	293.3±4.11	282.1±2.69	285.1±4.14	275.4±3.58** (↓6.1%)
	LD 14	305.4±3.62	296.7±3.31	301.3±3.90	292.6±3.65
	LD 21	297.3±3.60	290.2±3.09	291.0±4.63	287.2±3.79
Mean food consumption (g/animal/day)	LD 0-7	36.4±1.32	47.0±3.32*[b]	44.7±5.15[b]	36.0±1.20
	LD 7-14	53.4±0.86	52.3±1.03	51.9±1.08	51.0±0.83
	LD 14-21	62.6±1.04	61.5±1.07	59.7±1.26	60.5±0.85

[a] Data obtained from page 39 from MRID 50130601. Values are mean ± standard error (n). Means for gestation period include only dams known to deliver pups (either alive or dead).
[b] Value not considered an accurate measure of food consumption, due to spillage
N = 20-30
* Statistically different from control, p<0.05.
** Statistically different from control, p<0.01.

3. **Test substance intake:** Mean daily intake of transfluthrin was calculated as mg test substance/kg body weight and is presented in Table 3. Intake was based on maternal food consumption and body weight data during the gestation and lactation periods.

TABLE 3. Mean (±SE) maternal test substance intake (mg/kg body weight/day) [a]

Period	Dose (mg/kg/day)		
	42.1	161	534
Gestation			
GD 6-13	44.8±1.64	170.8±5.36	1179.0±128.14[b]
GD 13-20	44.0±1.41	164.6±4.19	582.9±19.04
Lactation			
LD 0-7	46.4±3.30	173.6±20.08	516.2±16.2
LD 7-14	39.7±0.80	157.5±2.95	543.0±5.7
LD 14-21	35.7±0.65	139.5±3.16	494.1±5.71

[a] Data obtained from page 40 from MRID 50130601. Values are mean ± standard error (n). Dietary concentrations were reduced during weeks 1-4 of lactation (by factors of 1.9, 2.3, and 2.8, respectively, based on estimated increases in feed consumption [g consumed/kg body weight/day] during lactation).
[b] Associated with observed food spillage and considered an unreliable measure of a.i. intake. This value was excluded from the mean average daily intake.

4. **Reproductive performance:** There was no treatment-related effect on reproductive performance (Table 4). The fertility index for the high-dose females was slightly lower (86.7%) compared to the controls but was within the range of historical controls (83.3-100%).

TABLE 4. Reproductive performance [a]

Observation	Dose (mg/kg/day)			
	Control	42.1	161	534
No. of animals co-housed[b]	30	30	30	30
No. of animals mated	30	30	30	30
Maternal Wastage:				
No. of dams not pregnant	0	2	3	4
No. of dams that delivered dead pups	1	2	2	0
No. of dams with pre-mature delivery	0	0	0	0
Mating index	100	100	100	100
Fertility index (No. of pregnant females/No. of inseminated females x 100)	100	93.3	90.0	86.7
No. of animals selected for study	22	21	20	23
No of animals evaluated for implants	22	21	18	22
Total No. of implantations	275	248	203	271
No. of implantations per dam	12.5±0.34 [12.5] (9.0-15.0)	11.8±0.39 [11.0] (10.0-16.0)	11.3±0.39 [11.0] (9.0-15.0)	12.3±0.43 [12.0] (9.0-17.0)
Mean (±SE) gestation duration (days)[c]	21.7±0.12 [22.0] (21.0-23.0)	21.6±0.11 [22.0] (21.0-22.0)	21.8±0.12 [22.0] (21.0-23.0)	21.5±0.11 [22.0] (21.0-22.0)

[a] Data obtained from page 41 from MRID 50130601.
[b] Number of animals assigned to each dietary level.
[c] Values are mean ± standard error, [median], and range
* Statistically different from control, $p<0.05$.
** Statistically different from control, $p<0.01$.

5. **Maternal postmortem results:** Not applicable to the present study.

B. OFFSPRING

1. **Viability and clinical signs:** Litter size and viability *(survival)* results from pups during lactation are summarized from the report in the following table. There was no effect on litter parameters and pup viability across all treatment levels.

TABLE 5. Litter size and viability [a]

Observation	Dose (mg/kg/day)			
	Control	42.1	161	534
Number of litters	22	21	20	23
Total number born	259	235	213	264
Total number missing	2	5	1	0
Litters with pups missing	2	4	1	0
Total number found dead	0	0	2[1]	1
Litters with pups found dead	0	0	2	1
Total number of pups cannibalized	1	0	1	0
Litter with pups cannibalized	1	0	1	0
Litter size:	11.8±0.36 [11.5] (9.0-15.0)	11.2±0.34 [11.0] (9.0-14.0)	10.6±0.33 [10.0] (9.0-13.0)	11.5±0.40 [12.0] (8.0-16.0)
Stillborn pups:				
Number	1	2	2	0
%	0.4	0.9	0.9	0.0
Mean ± SE	0.0±0.05	0.1±0.10	0.1±0.10	0.0±0.0
[Median]	[0.0]	[0.0]	[0.0]	[0.0]
(Range)	(0.0-1.0)	(0.0-2.0)	(0.0-2.0)	(0.0-0.0)
Mean number of viable pups				
Birth	12	11	11	11
Day 4 (Pre-cull)[b]	12	11	10	11
Day 4 (Post-cull)[c]	8	8	8	8
Day 21	8	8	8	8
Live birth index	99.6±0.41 [100.0] (91-100)	99.0±0.95 [100.0] (80-100)	99.1±0.91 [100.0] (82-100)	100.0±0.00 [100.0] (100-100)
Viability index	99.3±0.45 [100.0] (92-100)	98.4±0.77 [100.0] (89-100)	98.9±0.73 [100.0] (89-100)	100.0±0.00 [100.0] (100-100)
Lactation index	99.4±0.57 [100.0] (87.5-100)	99.4±0.60 [100.0] (87.5-100)	98.8±0.86 [100.0] (87.5-100)	99.5±0.54 [100.0] (87.5-100)

[a] Data obtained from page 42 in MRID 50130601.

[b] Before standardization (culling). Values are mean ± standard error.

[c] After standardization (culling).

There were no treatment-related clinical observations during lactation or post-weaning. All clinical observations were either isolated findings, occurred at similar incidences in treated groups and the control.

2. **Body weight:** For the high-dose females, pup body weights were statistically decreased on PND 11 (9%), but were not adversely affected at any other time point. The pup body weights

for all of the other treated groups were comparable to the controls. Selected mean pre-weaning pup body weight data are presented in Table 6.

TABLE 6. Mean (±SE) pre-weaning pup body weights (g) [a]

Postnatal day	Dose (mg/kg/day)							
	Control	42.1	161	534	Control	42.1	161	534
	Males				Females			
1	5.9±0.09	5.9±0.11	6.0±0.08	5.8±0.11	5.6±0.10	5.6±0.11	5.7±0.09	5.5±0.11
4 b	9.8±0.26	9.7±0.34	10.1±0.21	9.3±0.24	9.5±0.29	9.3±0.30	9.8±0.21	9.0±0.22
4 c	9.8±0.25	9.7±0.33	10.1±0.20	9.3±0.25 (↓5.1%)	9.5±0.30	9.3±0.31	9.8±0.22	9.0±0.23 (↓5.3%)
11	25.4±0.51	24.4±0.76	25.0±0.60	23.6±0.45 * (↓7.1%)	25.1±0.57	23.4±0.70	24.6±0.66	22.9±0.42* (↓8.8%)
17	39.7±0.71	37.6±0.97	38.5±0.85	37.0±0.62 * (↓6.8%)	38.6±0.68	36.9±0.95	37.4±0.84	36.1±0.44* (↓6.5%)
21	50.3±0.99	48.1±1.34	48.7±1.13	46.3±0.74 * (↓8.0%)	48.8±0.98	46.4±1.18	47.3±1.17	45.2±0.65* (↓7.4%)

[a] Data obtained from page 44 of MRID 50130601. Values are mean ± standard error.
[b] Before standardization (culling).
[c] After standardization (culling).
* Statistically different from control, $p<0.05$

Offspring post-weaning body weights were not affected by treatment. Selected mean post-weaning offspring body weight data are presented in Table 7.

TABLE 7. Mean (±SD) post-weaning pup body weights (g) [a]

Postnatal day[b]	Dose (mg/kg/day)							
	Control	42.1	161	534	Control	42.1	161	534
	Males				Females			
28	81.9±7.8	78.6±9.0	80.0±6.5	76.8±8.5	80.4±6.8	77.1±7.7	77.4±6.4	74.8±6.1
35	130.5±11.0	126.3±12.3	127.1±9.7	124.1±11.7	118.2±8.8	114.4±8.9	116.0±7.9	112.3±7.1
42	179.4±14.4	172.1±15.1	174.1±11.4	171.0±12.9	143.3±9.4	138.6±8.4	141.7±8.7	137.3±8.4
49	225.2±18.7	214.2±19.0	218.0±13.8	214.4±14.9	160.7±11.3	156.0±9.9	158.8±10.8	155.2±9.6
56	272.5±20.0	261.1±20.9	265.1±15.9	260.8±17.2	181.7±13.0	175.6±11.5	179.5±12.5	176.0±11.6
63	310.0±23.0	296.8±22.4	302.9±18.5	299.1±18.1	193.2±12.6	188.0±11.9	190.7±13.7	188.4±12.3
70	339.9±26.2	323.5±24.8	327.5±20.6	325.5±19.9	204.2±13.1	196.9±12.6	200.8±13.3	199.3±13.5

[a] Data obtained from page 44 of MRID 50130601. Values are mean ± standard deviation.
[b] Actual days of measurements occurred within the week of PND 28, 35, 42, 49, 56, 63,70
* Statistically different from control, $p<0.05$

3. Developmental landmarks

a. **Sexual maturation:** There were no treatment-related effects on sexual maturation for either sex; the ages for onset of balanopreputial separation and vaginal patency were comparable to the control animals for all treated groups.

TABLE 8. Mean (±SE) age of sexual maturation (days) [a]

Parameter	Dose (mg/kg/day)			
	Control	42.1	161	534
Preputial separation (males)	42.8±0.23	43.1±0.31	43.2±0.30	43.7±0.27
Vaginal opening (females)	31.8±0.29	32.2±0.36	31.8±0.34	31.8±0.32

[a] Data obtained from page 45 in MRID 50130601. Values are mean ± standard error (N)

b. **Physical landmarks:** The age of onset for surface righting was not affected by treatment at any dietary level. Additionally, pupil constriction in response to a penlight was apparent in all control and treated pups on PND 21.

4. Behavioral assessments

a. **Functional observational battery (FOB):** There were no treatment-related differences in males or females at any dietary level. Two control dams were sacrificed on LD 4 because their litter was insufficient for purposes of the study. One high-dose male was inadvertently not tested on PND 35; however, the remaining sample size (19-20/sex) was considered sufficient to establish compound-related effects. All findings were considered incidental and unrelated to treatment.

c. **Motor activity:** One mid-dose male pup was found dead and one mid-dose female and high-dose female were sacrificed since they had been mis-sexed at birth. One dam was sacrificed because the litter was insufficient for purposes of the study. However, the remaining sample size (19-20/sex) was considered sufficient.

There were no compound-related effects on motor or locomotor activity in males or females at any dietary level. Total motor and locomotor activity data are presented in Table 9 and 10. For motor activity, habituation was evident in both sexes at all ages, as indicated by the progressive decrease in activity over the test session. For locomotor activity, habituation was apparent in both sexes at most ages, except for PND13 when locomotor activity levels were generally low and variable during the first intervals.

TABLE 9. Mean (±S.D.) motor activity data (total activity counts for session) [a]

Test Day	Dose (mg/kg/day)			
	Control	42.1	161	534
Males				
PND 13	59±58	56±40	53±54	46±37
PND 17	156±123	186±132	197±96	182±153
PND 21	331±150	279±100	281±117	339±89
PND 60	564±149	513±118	564±88	524±102
Females				
PND 13	50±36	67±58	58±43	45±53
PND 17	143±82	167±117	174±113	153±100
PND 21	299±124	305±83	289±92	250±91
PND 60	663±192	722±188	754±161	732±200

[a] Data obtained from page 47 in MRID 50130601

TABLE 10. Mean (±S.D.) locomotor activity data (total activity counts for session) [a]

Test Day	Dose (mg/kg/day)			
	Control	42.1	161	534
Males				
PND 13	8±12	4±5	5±5	4±3
PND 17	32±30	52±41	52±32	40±44
PND 21	103±49	78±31	83±34	96±32
PND 60	387±127	347±105	386±67	351±91
Females				
PND 13	6±6	5±5	4±3	3±2
PND 17	36±29	43±40	39±28	33±29
PND 21	90±35	92±24	87±22	74±30
PND 60	428±128	502±170	480±92	437±121

[a] Data obtained from page 48 in MRID 50130601

d. <u>Auditory startle reflex habituation</u>: One control female (0120 06) was inadvertently miss-sexed and sacrificed on PND 27, so the PND 23 data were removed from the analysis. Additionally, one low-dose female (1114 06) was inadvertently not tested on PND 23. The remaining sample size for control and low-dose females (19 animals) was considered sufficient to establish whether there were compound-related effects.

The amplitude of the startle response increased with age for both sexes. The average response amplitude was 37 g and 222 g for control males and 37 g and 106 g for control females for PND 23 and 60 (±2 days), respectively. Response amplitude decreased over the course of the test session for both sexes, indicating that habituation occurred.

Startle amplitude, latency, and habituation were not affected by treatment at any dietary level, on any test occasion. There were a few statistical differences from control for mid- and high-dose females on PND 23 and 60 but these were not considered to be related to treatment since there was no dose response relationship, they were only observed in one sex, habituation was not affected, and the findings were inconsistent.

The amplitude and habituation data are presented in Tables 11a-c.

TABLE 11a. Mean (±SD) overall (Blocks 1-5) acoustic startle peak amplitude (g) and latency to peak (msec)[a]

Dose (ppm)	Parameter	Males		Females	
		PND 23	PND 60	PND 23	PND 60
Control	Peak Amp.	37±15	222±110	37±13	106±60
	Latency	37±4	39±3	36±2	41±3
42.1	Peak Amp.	32±15	204±139	31±12	87±55
	Latency	36±2	39±2	36±4	40±3
161	Peak Amp.	41±16	198±102	38±16	143±79
	Latency	35±2	40±2	38±4	39±4
534	Peak Amp.	29±14	187±116	28±11	88±61
	Latency	38±4	39±2	38±5	41±4

[a] Data were obtained from Text Table 14-15, pages 51-52 from MRID 50130601; n=19-20.

TABLE 11b. Mean (±SD) interval acoustic startle peak amplitude (g) and latency to peak (MSEC) in F_1 male rats[a]

Dose (ppm)	Parameter	Block 1	Block 2	Block 3	Block 4	Block 5
PND 23						
Control	Peak Amp.	39±17	42±19	39±17	36±17	30±16
	Latency	39±17	37±6	36±4	36±5	37±4
42.1	Peak Amp.	37±18	36±18	34±14	30±15	26±13
	Latency	36±3	35±2	35±3	36±3	36±4
161	Peak Amp.	40±18	44±18	42±17	41±18	39±19
	Latency	36±4	35±3	35±3	35±3	35±2
534	Peak Amp.	35±15	32±16	29±14	26±14	26±14
	Latency	38±6	39±6	37±5	38±7	38±5
PND 60						
Control	Peak Amp.	284±154	278±142	234±138	178±88	136±75
	Latency	40±2	39±2	38±3	38±4	38±5
42.1	Peak Amp.	268±161	219±145	196±155	168±139	169±131
	Latency	42±3	39±3	37±3	38±3	37±3
161	Peak Amp.	242±123	226±135	197±117	170±99	153±85
	Latency	42±3	40±3	38±3	39±2	39±4
534	Peak Amp.	245±149	222±140	189±133	152±98	128±105
	Latency	41±3	40±4	39±3	38±3	38±3

[a] Data were obtained from Text Table 14-15, pages 51-52 from MRID 50130601; n=19-20; 10 trials/block

TABLE 11c. Mean (±SD) interval acoustic startle peak amplitude (g) and latency to peak (MSEC) in F_1 female rats[a]

Dose (ppm)	Parameter	Block 1	Block 2	Block 3	Block 4	Block 5
PND 23						
Control	Peak Amp.	39±14	42±18	37±14	36±14	33±14
	Latency	39±3	35±2	37±4	36±3	36±3
42.1	Peak Amp.	33±12	32±15	32±12	29±14	29±12
	Latency	37±5	37±5	35±4	36±5	36±6
161	Peak Amp.	40±12	39±17	39±17	38±17	32±17
	Latency	40±6	39±7	37±6	37±4	36±4
534	Peak Amp.	31±14	30±15	30±14	24±10*** (↓33.3%)	24±11
	Latency	38±7	40±8	38±6	38±6	36±6
PND 60						
Control	Peak Amp.	123±75	153±98	116±95	75±45	61±35

Dose (ppm)	Parameter	Block 1	Block 2	Block 3	Block 4	Block 5
	Latency	43±6	40±3	39±6	41±5	41±6
42.1	Peak Amp.	105±65	111±84	96±83	71±45	54±32
	Latency	42±4	40±4	40±5	40±4	40±5
161	Peak Amp.	140±70	166±97	165±104	139±99*** (↑85.3%)	104±72*** (↑70.5%)
	Latency	41±5	38±4	39±5	39±5	39±4
534	Peak Amp.	104±64	113±92	92±73	69±46	64±48
	Latency	40±6	41±5	40±3	41±6	41±5

[a] Data were obtained from Text Table 14-15, pages 51-52 from MRID 50130601; n=19-20; 10 trials/block
*** Significantly different from controls at p<0.05

e. <u>Learning and memory testing</u>: There were no compound-related effects for either sex at any dose in the passive avoidance or water maze tests (Tables 12 and 13). One control female (0111 11) was tested for acquisition but was not tested for retention. One mid-dose female (2116 08) failed acquisition but was tested for retention, so these data were not included. The remaining sample size was considered sufficient to establish whether there were compound-related effects.

In the passive avoidance test, acquisition and retention was evident for controls as a marked increase in latency to cross for the second trial (an average of 180 seconds for both males and females) compared to the first trial (an average of 55 and 33 seconds for males and females, respectively). No control animals crossed within the 180-sec limit after the first trial. Any animal that failed to establish acquisition or failed to cross during the first two trials were not tested for retention. On the second test occasion, retention was evident in control males and females by a reduced average number of trials-to-criterion (2.2 and 2.6 trials for males and females, respectively) compared to the first (2.9 trials, each). These comparisons were not subjected to statistical analysis.

In the water maze test, acquisition was evident in both sexes as a progressive decrease in the average time to escape over successive trials. For males the average trial duration (time to escape) decreased from the first trial to the second trial (an average 20.8 vs. 18.0 sec, respectively). By comparison for females, the average trial duration did not decrease but remained nearly unchanged from the first trial to the second trial (an average of 18.0 vs. 18.5 sec, respectively). This could be due to the performance of six control females that took longer to escape for trial 2 compared to trial 1. However, further reductions were evident in the time to escape for subsequent trials for both sexes (e.g., an average 7.6 and 5.3 sec for males and females, respectively, for the fifth trial). Retention was evident as a reduction in the number of trials-to-criterion and a shorter trial duration for the first trial compared to the first trial of acquisition.

TABLE 12. Passive avoidance performance at PND 23 and 30 offspring (mean ± S.D.) [a]

Session/Parameter		Dose (mg/kg/day)			
		Control	42.1	161	534
Males					
Session 1	Trials to criterion	2.9±0.3	3.3±1.0	3.6±2.0	3.0±0.0
	Latency trial 1 (sec)	55.2±49.5	33.7±22.8	49.8±52.9	54.3±42.9
	Latency trial 2 (sec)	180.0±0.0	180.0±0.0	175.6±17.8	180.0±0.0
	Failed to Meet Criterion	0 (0%)	0 (0%)	0 (0%)	0 (0%)
	Failed to Cross During Learning Phase	1 (6%)	0 (0%)	1 (6%)	0 (0%)
Session 2	Trials to criterion	2.2±0.8	2.1±0.3	2.1±0.4	2.2±0.5
	Latency trial 1 (sec)	176.2±14.6	170.8±36.8	161.2±49.7	175.4±18.5
	Latency trail 2 (sec)	180.0±0.0	180.0±0.0	180.0±0.0	176.9±12.5
Females					
Session 1	Trials to criterion	2.9±0.3	2.9±0.3	3.0±0.4	3.1±0.3
	Latency trial 1 (sec)	33.3±44.8	43.0±56.0	44.2±48.6	53.3±44.0
	Latency trial 2 (sec)	180.0±0.0	180.0±0.0	171.8±32.9	175.1±19.6
	Failed to Meet Criterion	0 (0%)	0 (0%)	0 (0%)	0 (0%)
	Failed to Cross During Learning Phase	1 (6%)	2 (12%)	1 (6%)	0 (0%)
Session 2	Trials to criterion	2.6±0.9	2.5±0.9	2.3±0.6	2.3±0.7
	Latency trial 1 (sec)	167.1±32.8	145.5±53.7	163.6±45.7	175.7±17.4
	Latency trial 2 (sec)	170.7±18.5	180.0±0.0	174.7±20.5	169.0±31.2

[a] Data extracted from Text Table 16 on page 53 of MRID 50130601.
N = 16-17
Trials to Criterion — Mean # Trials per Group ± S.D.
Latency to Trial 1 — Mean Session 1 duration (seconds) per Croup ± S.D.
Latency to Trial 2 = Mean Session 2 duration (seconds) per Group ± S.D.
Failed to Meet Criterion = Number of Animals that received the shock but did not demonstrate acquisition.
Failed to Cross = Number of Animals that never received the shock.

TABLE 13. Water maze performance in PND 60 (±2 days) and 67 (±2 day) offspring (mean ± S.D.) [a]

Session/Parameter		Dose (mg/kg/day)			
		Control	**42.1**	**161**	**534**
Males					
Session 1	Latency trial 1 (sec)	20.80±13.1	20.1±14.4	19.1±14.2	29.6±20.2
	Errors trial 1	0.8±0.9	0.7±1.0	0.6±0.7	1.1±1.2
	Latency trial 2 (sec)	18.0±13.8	17.2±14.3	14.5±12.7	24.1±14.9
	Errors trial 2	0.4±0.6	0.4±0.5	0.4±0.5	0.4±0.7
	Trials to criterion	7.6±3.0	8.2±2.7	6.4±1.6	6.9±1.8
	Failed to learn	1 (6%)	0 (0%)	0 (0%)	0 (0%)
Session 2	Latency trial 1 (sec)	12.7±10.2	7.6±6.8	10.4±7.0	8.9±7.6
	Errors trial 1	0.5±0.6	0.1±0.3	0.4±0.6	0.4±0.7
	Latency trial 2 (sec)	7.7±5.9	5.0±3.4	3.9±1.2	5.1±2.6
	Errors trial 2	0.3±0.6	0.1±0.	0.0±0.0	0.0±0.0
	Trials to criterion	6.1±1.8	5.4±1.1	5.4±0.5	5.5±1.0
Females					
Session 1	Latency trial 1 (sec)	18.0±8.9	15.3±8.7	18.3±13.4	22.6±15.6
	Errors trial 1	0.9±0.7	0.7±0.8	0.8±0.8	0.9±0.7
	Latency trial 2 (sec)	18.5±13.1	14.8±13.6	8.1±4.3	16.4±12.3
	Errors trial 2	0.9±0.8	0.9±1.1	0.3±0.6	0.8±1.0
	Trials to criterion	7.9±2.3	7.1±1.7	6.7±1.7	8.5±3.2
	Failed to learn	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Session 2	Latency trial 1 (sec)	9.7±6.7	7.9±7.3	6.9±5.0	10.0±8.1
	Errors trial 1	0.5±0.7	0.3±0.7	0.1±0.3	0.5±0.7
	Latency trial 2 (sec)	4.9±3.3	5.5±7.6	4.5±1.9	5.1±3.5
	Errors trial 2	0.0±0.0	0.1±0.5	0.1±0.3	0.1±0.3
	Trials to criterion	5.6±0.8	6.4±2.2	6.4±2.2	6.6±1.9

[a] Data obtained from *page 58* in MRID 50130601.
N = 15-16; values for rats who failed to learn during session 1 were not included in means for session 2.

5. Postmortem results

a. Brain weights: Mean brain weight data are presented in Table 14. There were no treatment-related effects on brain weight for either sex at PND 21 or 75. While the relative brain weight for the low-dose, non-perfused PND 75 males was statistically increased, this was the result of the decreased terminal body weight and was not considered to be the result of an effect on brain weight.

TABLE 14. Mean (±SD) brain weights for PND 21 and 75 males and females.

Parameter	Dose (mg/kg/day)			
	Control	42.1	161	534
Males				
Day 21 (Perfused)				
Terminal body weight (g)	50.4±5.5	47.5±8.2	47.3±3.8	46.8±2.7
Brain weight, fixed (g)	1.411±0.077	1.399±0.113	1.399±0.053	1.411±0.064
Brain (fixed)-to-body weight ratio (%)	2.829±0.314	3.011±0.462	2.983±0.359	3.024±0.201
Termination (PND 75±5 – Perfused)				
Terminal body weight (g)	354.1±25.5	337.0±36.4	319.1±26.9	329.7±35.4
Brain weight, fixed (g)	1.880±0.059	1.837±0.094	1.830±0.078	1.819±0.060
Brain (fixed)-to-body weight ratio (%)	0.533±0.028	0.551±0.062	0.576±0.037	0.557±0.058
Termination (PND 75±5 – Non-Perfused)				
Terminal body weight (g)	358.7±20.8	309.9±26.7* (↓13.6%)	324.6±21.3* (↓9.5%)	326.6±27.5* (↓8.9%)
Brain weight, fresh (g)	1.999±0.092	1.959±0.086	1.963±0.104	1.926±0.098
Brain (fresh)-to-body weight ratio (%)	0.559±0.033	0.637±0.066* (↑13.9%)	0.606±0.034	0.593±0.054
Females				
Day 21 (Perfused)				
Terminal body weight (g)	48.6±4.1	47.4±4.2	47.7±5.3	45.9±4.8
Brain weight, fixed (g)	1.372±0.077	1.344±0.057	1.346±0.069	1.340±0.051
Brain (fixed)-to-body weight ratio (%)	2.837±0.228	2.848±0.227	2.847±0.282	2.944±0.277
Termination (PND 75±5 – Perfused)				
Terminal body weight (g)	198.2±11.4	193.7±15.0	199.6±8.9	206.7±19.0
Brain weight, fixed (g)	1.697±0.079	1.732±0.071	1.735±0.050	1.708±0.053
Brain (fixed)-to-body weight ratio (%)	0.858±0.048	0.897±0.054	0.870±0.038	0.831±0.061
Termination (PND 75±5 – Non-Perfused)				
Terminal body weight (g)	201.3±15.7	204.4±12.6	208.9±19.6	192.9±11.7
Brain weight, fresh (g)	1.813±0.068	1.771±0.103	1.854±0.080	1.782±0.064
Brain (fresh)-to-body weight ratio (%)	0.904±0.052	0.869±0.061	0.894±0.082	0.927±0.069

[a] Data obtained from page 58 in MRID 50130601.
N = 10
* Statistically different from control, p<0.05
** Statistically different from control, p<0.01

b) Neuropathology

1. **Macroscopic examination:** There were no compound-related necropsy findings in animals that were either found dead or sacrificed on PND 21 or at study termination. All findings were considered to be incidental and unrelated to treatment.

2. **Microscopic examination:** There were no statistically significant differences in

micropathology brain measurements in high-dose males at PND 21 or 75. For the PND 75 females, the hippocampus measurement (1.43 mm) in the high-dose was statistically decreased compared to the controls (8% decrease) and was smaller than the concurrent (1.56 mm) and historical (1.48-1.76 mm) controls. The frontal cortex thickness was statistically increased for all treated groups but this finding was not attributed to treatment due to the lack of a dose response. In addition, the group mean for the control females was below the historical control range (1.66-2.00 mm), and the group means for the dietary levels were in the historical control range. The qualitative histopathological findings as well as the morphometric evaluation are presented in Table 15.

TABLE 15. Histopathology findings [a]

Parameter	Dose (mg/kg/day)			
	Control	42.1	161	534
Males				
Gross Measurements				
Day 21				
Ant/Post Cerebrum Length (mm)	13.62±0.28	13.73±0.28	13.63±0.31	13.75±0.24
Ant/Post Cerebellum (mm)	7.14±0.25	7.08±0.39	7.16±0.34	7.21±0.41
Termination (PND 75±5 – Perfused)				
Ant/Post Cerebrum Length (mm)	14.90±0.31	14.61±0.25	14.67±0.28	14.73±0.45
Ant/Post Cerebellum (mm)	7.79±0.36	7.78±0.40	7.66±0.42	7.64±0.32
Microscopic Measurements				
Day 21				
Frontal Cortex (mm)	1.726±0.005	--	--	1.720±0.003
Parietal Cortex (mm)	1.820±0.004	--	--	1.844±0.011
Caudate Putamen (mm)	2.876±0.007	--	--	2.828±0.009
Hippocampal Gyrus (mm)	1.522±0.041	--	--	1.540±0.020
Cerebellum (mm)	4.903±0.037	--	--	4.855±0.026
Termination (PND 75±5 – Perfused)				
Frontal Cortex (mm)	1.705±0.016	--	--	1.658±0.008
Parietal Cortex (mm)	1.894±0.011	--	--	1.858±0.013
Caudate Putamen (mm)	3.304±0.015	--	--	3.243±0.029
Hippocampal Gyrus (mm)	1.803±0.015	--	--	1.736±0.012
Cerebellum (mm)	4.849±0.263	--	--	4.638±0.210
Females				
Gross Measurements				
Day 21				
Ant/Post Cerebrum Length (mm)	13.58±0.37	13.60±0.17	13.47±0.28	13.48±0.27
Ant/Post Cerebellum (mm)	7.28±0.37	6.86±0.38* (↑5.8%)	7.20±0.29	7.04±0.25
Termination (PND 75±5 – Perfused)				
Ant/Post Cerebrum Length (mm)	14.90±0.31	14.61±0.25	14.67±0.28	14.73±0.45
Ant/Post Cerebellum (mm)	7.79±0.36	7.78±0.40	7.66±0.42	7.64±0.32
Microscopic Measurements				
Day 21				
Frontal Cortex (mm)	1.706±0.007	--	--	1.722±0.007
Parietal Cortex (mm)	1.789±0.007	--	--	1.805±0.005
Caudate Putamen (mm)	2.876±0.011	--	--	2.852±0.008
Hippocampal Gyrus (mm)	1.596±0.004	1.374±0.013* (↓13.9%)	1.509±0.016 (↓5.5%)	1.517±0.003* (↓4.9%)
Cerebellum (mm)	4.596±0.082	--	--	4.609±0.077
Termination (PND 75±5 – Perfused)				
Frontal Cortex (mm)	1.578±0.003	1.665±0.001* (↑5.5%)	1.659±0.005* (↑5.1)	1.638±0.005* (↑3.8%)
Parietal Cortex (mm)	1.754±0.004	--	--	
Caudate Putamen (mm)	3.157±0.003	--	--	
Hippocampal Gyrus (mm)	1.561±0.008	1.598±0.011	1.481±0.013	1.428±0.014* (↓8.5%)
Cerebellum (mm)	4.517±0.055	--	--	

[a] Data obtained from page 60 in MRID 50130601.
N = 10
* Statistically different from control, $p<0.05$

** Statistically different from control, $p<0.01$

III. DISCUSSION and CONCLUSIONS

A. **INVESTIGATORS' CONCLUSIONS:** Technical-grade transfluthrin was administered via the diet from gestation day (GD) 6 through lactation day (LD) 21 to mated female Wistar rats, at nominal concentrations of 0, 500, 2000 and 7000 ppm. The offspring were evaluated using detailed clinical observations, body weight, food consumption, developmental landmarks for sexual maturation, automated measures of activity (figure-eight maze), auditory startle habituation, learning and memory (passive avoidance and a water maze task), and an ophthalmic examination. Tissues were collected for morphometry (brain) and microscopic examination on PND 21 (brain) and at study termination (brain, an assortment of additional neural tissues, and skeletal muscle).

In summary, the following observations were noted.

General
The average daily intake of active ingredient by the dams during gestation and lactation was 0, 42.1 161 and 534 mg/kg/day. There was no effect on reproduction parameters at any dietary level.

Maternal
At 500 ppm, there were no adverse treatment-related findings during gestation or lactation.

At 2000 ppm, there were no adverse treatment-related findings during gestation or lactation.

At 7000 ppm, there were no adverse treatment-related findings during gestation or lactation. There was a statistical increase in food consumption during GD 6-13. Body weight gain during gestation was slightly reduced compared to the control animals. Lastly, body weight was statistically reduced on LD 0, 4 and 7. These differences from control are ascribed to palatability and therefore not considered adverse effects.

Offspring
At 500 ppm, there were no treatment-related findings.

At 2000 ppm, there were no treatment-related findings.

At 7000 ppm, body weight was statistically decreased in females on PND 11. Body weight gain was statistically decreased on PND 4-11 in females and males and females, combined. Also, body weight gain was statistically decreased on PND 4-21 in males, females and males and females, combined. Thus, the offspring LOEL is 534 mg/kg bw/day, based on decreased body weight in PND 11 females, reduced body weight gain on PND 4-11 (females and males and females, combined) and on PND 4-21 (males, females and males and females, combined) and the NOEL is 161 mg/kg bw/day. The reduced body weight for high-dose pups was associated with decreased bodyweight in the dams, compared to controls.

B. **REVIEWER COMMENTS:** Maternal toxicity: No treatment related deaths were observed in males and females in the P-generation. Compound-related clinical signs were not evident at any dietary level during gestation or lactation periods. There were no treatment-related effects to body weight, food consumption, and reproductive performance or parameters.

The maternal LOAEL for transfluthrin was not established. The maternal NOAEL is 7000 ppm (equivalent to 534 mg/kg/day).

Offspring toxicity: There was no effect on litter parameters and pup viability across all treatment levels. Offspring body weights during lactation were statistically decreased in the high-dose females and males at PND 11, 17, and 21 (>5%). There was statistically significant decrease in the average number of urine pools during open field observations for low- and high- dose females on PND 21 and for mid-dose females on PND 45 but was not considered treatment-related because the differences were minimal and within the range of historical controls. There were no compound-related effects on measures of motor or locomotor activity in males or females at any dietary level.

There was no treatment-related effect on absolute brain weight for either sex on PND 21 or 75. While there was a statistically significant increase in relative to body brain weight for the non-perfused PND 75 males, this was considered to be due to the decreased absolute body weight and not a sign of a compound-related effect on brain weight. There were no compound-related necropsy findings in animals that were either found dead or sacrificed on PND 21 or at study termination. However, the hippocampus measurement (1.4 mm) in the high-dose females was statistically decreased compared to the controls (8% decrease) on PND 75 and was smaller than the concurrent (1.56 mm) and historical (1.48-1.76 mm) controls but was not considered toxicologically adverse due to no effects at PND 21 and no functional effects (learning and memory). The frontal cortex thickness was statistically increased at all dietary levels but were not attributed to treatment due to the lack of a dose response, the group mean for the control females was below the historical control range, and the group means for the dietary levels were in the historical control range.

The ages for onset of balanopreputial separation, vaginal patency, sexual maturation, onset for surface righting and pupil constriction were not affected by treatment at any dietary level.

The offspring LOAEL for transfluthrin 7000 ppm (equivalent to 534 mg/kg/day) based on decreased pup body weight in males and females (>5%).

C. STUDY DEFICIENCIES*:* The study was conducted with a few deviations to the guidelines outlined in the 1999 Data Call-In (DCI) Notice issued for several organophosphorus insecticides. The modifications include: (1) extend exposure to lactation / postnatal day 21 (rather than day 11) and (2) evaluate brains from 21-day-old (rather than 11-day-old) animals for morphometry and micropathology using (3) a sample size of 10 (rather than six) per sex per dietary level. These design elements are consistent with the draft OECD guideline for a Developmental Neurotoxicity Study (TG 426).

Motoractivity data were provided; however, the agency has concerns given the low motor activity counts and the high variability of PND13.